

Application No. 09/757,673

Response to Notice of Non-Responsive Amendment dated January 28, 2005

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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claims 1-6. Canceled.

7 (Previously Presented). A method for expressing a transgene in a skeletal muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV is at least as free of contamination with a helper virus as is obtained by subjecting the rAAV to four rounds of cesium chloride gradient centrifugation and wherein the transgene is expressed in the cell.

8 (Previously Presented). The method according to claim 7, wherein the transgene encodes a secretable protein.

9 (Previously Presented). The method according to claim 8, wherein the protein is selected from the group consisting of apoE, β -interferon, insulin, erythropoietin, growth hormone, and parathyroid hormone.

10 (Previously Presented). The method according to claim 7, wherein the rAAV consists essentially of, from 5' to 3', 5' AAV inverted terminal repeats (ITRs), a heterologous promoter, the transgene, a polyadenylation sequence, and 3' AAV ITRs.

Claim 11. Canceled.

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12 (Previously Presented). A recombinant adeno-associated virus (rAAV) comprising sequences encoding factor IX and regulatory control sequences which permit expression of factor IX in a cell, wherein the rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation.

13 (Previously Presented). A composition comprising a physiologically compatible carrier and a recombinant adeno-associated virus (AAV) comprising sequences encoding factor IX and regulatory control sequences which permit expression of factor IX in a cell, wherein the rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation.

14 (Previously Presented). The composition according to claim 13, wherein said composition comprises about 1×10^8 to about 5×10^{11} particles of the recombinant adeno-associated virus.

15 (Previously Presented). The composition according to claim 14, wherein said composition comprises at least 10^9 particles of the recombinant adeno-associated virus.

16 (Previously Presented). The composition according to claim 13, wherein the composition comprises 10^{12} to 10^{13} genomes of the recombinant adeno-associated virus per milliliter carrier.

17 (Previously Presented). The composition according to claim 13, wherein said composition is formulated for intramuscular injection.

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18 (Previously Presented). A method of delivering a transgene to a mammal comprising the step of:

administering intramuscularly to a mammal a composition comprising a biologically compatible carrier and a recombinant adeno-associated virus (rAAV) comprising a transgene encoding a secretable protein operably linked to sequences which control expression thereof, wherein said rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said rAAV to four rounds of cesium chloride gradient centrifugation, whereby the protein is secreted from rAAV-transduced muscle cells.

19 (Previously Presented). The method according to claim 18, wherein the composition comprises about 1×10^8 to about 5×10^{11} particles of the rAAV.

20 (Previously Presented). The method according to claim 18, wherein the composition comprises at least 10^9 particles of the rAAV.

21 (Previously Presented). The method according to claim 18, wherein the composition comprises 10^{12} to 10^{13} genomes of the rAAV per milliliter carrier.

22 (Previously Presented). The method according to claim 18, further comprising the step of monitoring expression of the transgene in the mammal.

23 (Previously Presented). The method according to claim 18, wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said rAAV to four rounds of cesium chloride centrifugation.

24 (Previously Presented). The composition according to claim 13, wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said rAAV to four rounds of cesium chloride centrifugation.

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25 (Previously Presented). A method of delivering a transgene to a mammal comprising the step of administering to a mammal intramuscularly a composition comprising a biologically compatible carrier and a helper-free recombinant adeno-associated virus (rAAV) comprising a transgene encoding a secretable protein operably linked to sequences which control expression thereof.

26 (Previously Presented). The method of claim 25 wherein the secretable protein is selected from the group consisting of Factor IX, apoE, β -interferon, insulin, erythropoietin, growth hormone, and parathyroid hormone.

27 (New). A method according to claim 7, wherein the rAAV is introduced in a composition that contains less than 1 infectious units of wild-type AAV per 10^9 rAAV.

28 (New). A composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) a 5' AAV inverted terminal repeat (ITR), (b) nucleic acid sequences encoding human apolipoprotein E (ApoE) operably linked to regulatory sequences which direct its expression, and (c) a 3' AAV ITR, and

wherein said recombinant AAV is at least as free of contamination with a helper virus as is obtained by subjecting the rAAV to four rounds of cesium chloride gradient centrifugation and wherein the transgene is expressed in the cell.

29 (New). The composition according to claim 28, wherein the composition contains less than 1 infectious unit of wild-type AAV per 10^9 AAV.

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30 (New). A method of delivering apolipoprotein E (ApoE) to a mammal with atherosclerosis, said method comprising the step of

administering to the mammal a composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) a 5' AAV inverted terminal repeat (ITR), (b) nucleic acid sequences encoding human apolipoprotein E (ApoE) operably linked to regulatory sequences which direct expression thereof and (c) a 3' AAV ITR, wherein the recombinant AAV is at least as free of contamination with a helper virus as is obtained by subjecting the rAAV to four rounds of cesium chloride gradient centrifugation and wherein the transgene is expressed in the cell

and wherein the ApoE in said composition is expressed in the mammal.

31 (New). The method according to claim 30, wherein the recombinant AAV contains less than 1 infectious unit of wild-type AAV per 10^9 AAV.

32 (New). A method for expressing a transgene in a muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV is free of contamination with immunogenic adenoviral helper and wherein the transgene is expressed in the cell.

33 (New). A method for expressing a transgene in a muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV is purified of adenoviral helper such that transgene is expressed in the absence of destructive inflammation caused by contaminating helper adenovirus.

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34 (New). A method for expressing a transgene in a muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV is purified of adenoviral helper such that transgene is expressed in the absence of destructive cytotoxic immune response against contaminating adenoviral antigens.

35 (New). A method for expressing a transgene in a muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV is purified of adenoviral helper such that transgene expression is prolonged due to the absence of an antibody response against contaminating adenoviral antigens.